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Aromatherapy as an environmental tool for improving the welfare of horses during the equine-assisted therapy sessions

Tágata Faccenda[1,2], Marcos Antonio de Oliveira[1], Rosangela Poletto[1]*

[1] Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, Campus Sertão, Sertão, RS, Brasil.
[2] Hotelaria Estância Riograndense, Sarandi, RS, Brasil.

ABSTRACT - The study aimed to assess the effects of pre-exposure to lavender aromatherapy on horse behavior in stalls and during therapy sessions at the Equine Therapy Center of Cavalo Crioulo/IFRS. Eight therapy horses underwent three control (water diffusion) followed by three aromatherapy trials (water + five drops of diffused lavender essential oil for 30 min) in the stalls. Each trial was on a separate day/week over six consecutive weeks. Total time spent (min) and frequency of behavior in the stalls (head in the window, standing by the diffuser, head down, agitated) and then during the 20-minute therapy sessions (balk, bite, lean on the embarkation, quit session, head-butts) were determined. Perception of treatment-blind handlers who prepared and guided the horses during the therapy sessions was assessed with a closed survey after they ceased. Compared with control trials, diffused lavender aromatherapy resulted in horses spending more time with their heads down (21.2±1.3 vs. 26.9±1.4 min, P = 0.004), lower proportion of agitated behavior (12.5% vs. 87.5%, P<0.0001) and fewer visits to the stall's window (29.2% vs. 65.2%, P = 0.003). The duration of head-butting was half post lavender exposure (0.14 vs. 0.06±0.01 min, P<0.05) and frequency of this behavior and biting the handlers during the sessions were approximately 2.5 times lower post-aromatherapy (P<0.05). According to most handlers (77%), horses were calmer than usual post-aromatherapy with no impediment to horse performance. Horses subjected to lavender essential oil diffused for 30 min before therapy sessions were calmer and easier to handle, suggesting that aromatherapy is a relevant and practical approach to improve horse welfare within animal-assisted therapy settings.

Keywords: behavior, equine, essential oil, human therapy, lavender, one-welfare

***Corresponding author:**
rosangela.poletto@sertao.ifrs.edu.br

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1. Introduction

Equine therapy is an animal-assisted therapy that employs the horse´s walking movements (equine-assisted therapy) as kinesiotherapy to the patient. The horse as a therapy aid promotes educational, physical, and psychological benefits to individuals at risk and to those with special needs or disabilities (ANDE-Brasil, 2023; Kaiser et al., 2006). To achieve this goal, special attention must be given to behavioral profile when selecting therapy horses (Mills and Nankervis, 2005; McDuffee et al., 2022). Animals that display normal species-appropriate behaviors, remain calm, respond to their handler's commands, and have good leg and hoof conformation are preferred (ANDE-Brasil, 2023). A positive

and safe human-horse interaction and relationship is essential for consolidating the benefits of the equine in rehabilitation programs (Kelly et al., 2021; Merkies and Franzin, 2021).

Management and handling practices applied to horses used in animal-assisted therapy must at least meet their basic needs to mitigate or prevent accidents and undesired behaviors (McDuffee et al., 2022; Mills and Nankervis, 2005). Practices considered as "correct or adequate" but, non-adaptable by horses (Broom, 1986; Young et al., 2012), such as prolonged stabling, reduced socialization with conspecifics, adverse handling, among others, may compromise the horse's physical and behavioral homeostasis (Mills and Nankervis, 2005; Waters et al., 2002). These, in turn, can negatively affect the progress of the equine-assisted therapy sessions. Thus, alternative and practical tools that can be environmentally employed for positively modulating problematic behaviors in horses are valuable for supporting human therapy.

Inhalational aromatherapy consists of diffusing essential oils into the animals´ environment, stimulating olfactory receptors and triggering neurophysiological effects throughout the limbic system (Aponso et al., 2020). Based on this underlying mechanism, aromatherapy has been used to modulate behavioral, emotional, and physical outcomes depending upon the type of essential oil used and its effects on the target individual (Morag, 2018). Due to its calming effect, diffusing lavender essential oil has been applied to treat anxiety and mitigate behavioral disorders in humans and animals (Aponso et al., 2020; Poutaraud et al., 2018). Furthermore, oral administration of lavender oil in mice has demonstrated analgesic and anti-inflammatory effects (Silva et al., 2015), emphasizing the oil´s therapeutic potential for horses used in equine-assisted therapy, which are often older animals of unknown previous health or handling history (Sousa et al., 2025).

A few studies have reported the application of aromatherapy to modulate equine behavioral and physiological responses (Ferguson et al., 2013; Heitman et al., 2018; Kosiara and Harrison, 2021). In horses, diffusing lavender essential oil in a transport trailer for 15 min was associated with reduced blood cortisol concentrations (Heitman et al., 2018). Similarly, exposing horses to lavender aromatherapy for 15 minutes resulted in lower heart and respiratory rates after they were subjected to sudden air horn noise (an acute stressor) blown twice for 15 seconds in an area adjacent to the horse stall (Ferguson et al., 2013).

For successful outcomes of the equine-assisted therapy, it is critical to be aware of the horse's behaviors (mainly undesired ones) and employ tools for minimizing any potential interference of them during the sessions (ANDE-Brasil, 2023). Based on available scientific evidence, no previous studies have assessed the effects of lavender aromatherapy on horses within the context of animal-assisted therapy sessions. Therefore, the aim of this study was to expose horses to lavender essential oil diffusion for 30 minutes in their individual stalls and assess the effects of the aromatherapy on their behavior while in the stalls and soon after, during the 20-minute equine-assisted therapy sessions. Furthermore, handlers' perceptions of therapy-horse behavioral performance during the sessions were evaluated at the end of the respective sessions.

2. Material and methods

2.1. Study site and animals

The study was carried out at the Equine Therapy Center of Cavalo Crioulo located at the Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul (IFRS) – Campus Sertão, Sertão, Rio Grande do Sul, Brazil (latitude 28°02'54" S, longitude 52°16'18" W). The average daily temperature during the experimental period (August through September 2019) was 22 °C. All *in vivo* experimental procedures were approved by the IFRS Ethical Committee for the Use of Animals (CEUA) protocol number 1052060819.

The Equine Therapy Center was founded in July 2012 and is well recognized in the region, conducting daily 20-min equine-assisted therapy sessions in both indoor and outdoor arenas, thus meeting

community demands in the North and High Uruguay regions of the state of Rio Grande do Sul. The eight therapy horses used in the center alternate according to the schedule established by each patient's therapy team. The indoor arena (Figure 1) was built according to recommendations from ANDE-Brasil (2023), the Brazilian Association of Equine Therapy, with an area of 150.50 m^2 covered with a 25-cm layer of medium sand, and wooden side walls for safety. A ramp for wheelchair accessibility was available in the indoor arena, along with structures in the surroundings, such as physical and educational features for patient interaction during sessions, and mirrors fixed to the walls. The outdoor arena (Figure 1) contained an area of 260.25 m^2 of ground covered by a layer of 15 to 20 cm of medium sand along its entire length for less impact on walking and human and animal comfort. It also had an access ramp for wheelchair users and public in general. Only the indoor arena was considered as an experimental area for this study.

Figure 1 - On the left, the indoor arena with the boarding and disembarkation ramp for practitioners shown in gray, and the equipment used in the equine-assisted therapy sessions. On the right, the outdoor arena of the Equine Therapy Center of the Crioulo Horse, IFRS-Campus Sertão, Sertão, RS, Brazil.

The activities developed in the equine-assisted therapy were guided by the horse's handler (a person familiar to the respective horse) in a calm and natural manner, while the patient, assisted by the health professionals, participated in the therapy session. The therapy session involved the patient following and interacting with the signs, figures, and obstacles displayed on the indoor walls and surroundings of the arena, and with the horse´s movement. The session was structured according to each horse's gait and adapted to the motor or physical limitations of each patient.

For this study, all the eight therapy horses used in the center, castrated male Crioulo mixed breed horses, aged between 8 and 23 years, were used in the experimental trials. The animals were donated to the Center at different times, so some have been at the Center and used as therapy horses in equine-assisted therapy sessions for eight years, while others for about one year.

Following the local daily routine, the animals were brought at 18:00 h to two individual but adjacent stalls that were familiar to all horses, where they stayed overnight or until 08:00 h. These stalls (10.5 m^2/stall) were made of masonry, separated between them by a 1.5 m high wall and the rest enclosed with a vertical metal mesh (enabling physical and visual contact between animals). The floor was made of dirt with a layer of sand covered by shavings. Each stall also contained a Dutch-style window (2.1 m high where 1.1 m is the door and 1 meter is the height of the window). Each stall also had a drinking trough (80 cm × 40 cm) with an automatic float to control the water level, located on the wall between the two stalls, which allowed access to the two animals, and a feeder (35 cm × 100 cm × 25 cm) with a hay holder installed above it for the supply of preserved forages (average 5 kg of Tifton hay supplied daily), located on the wall opposite the trough.

All animals received the same feeding regime and diet established by the attending veterinarian, according to the age and body condition of each horse. Feeding was provided in the individual stalls in the early morning and consisted of pellet ration (Alisul-Supra Alimentos S.A., Carazinho, RS) and white oat grain. In the stalls, horses had *ad libitum* access to water and mineral salt blocks. After feeding and following the schedule of the center, the horses not participating in the therapy sessions of the day were released in the pasture paddocks nearby the center. They were outdoors until late afternoon and were then housed in their respective stalls overnight.

2.2. Equine-assisted therapy sessions

The sessions carried out at the Equine Therapy Center of Cavalo Crioulo took place in both the morning and afternoon periods and consisted of different practices, depending on the pathology or condition of the patient. However, based on the availability of researchers, only the afternoon sessions and those that took place in the indoor arena were recorded for this study. After therapy sessions of the day, trial horses were released in the pasture paddocks with conspecifics.

According to the therapy session schedule of the center, and as mentioned in the section 2.1, horses were randomly distributed so that two out of the eight animals were evaluated per afternoon session, totaling four days per trial week. In this order, the trials per each experimental horse lasted for six continuous weeks (3 weeks/treatment). The control treatment was carried out in the first three weeks and the lavender diffusion treatment was carried out in the last three weeks, meaning that every therapy horse was its own control.

Initially, each horse was subjected to experimental treatments in the stalls (described in detail below). Thereafter, the animals were taken to prepare for the session where they were brushed, the hooves cleaned, the saddle, girth and reins installed to start the equine-assisted therapy session.

During the sessions, the animals were led by the halter, by handlers who had the animal as their focus and familiar with both the horses and the therapy procedures.

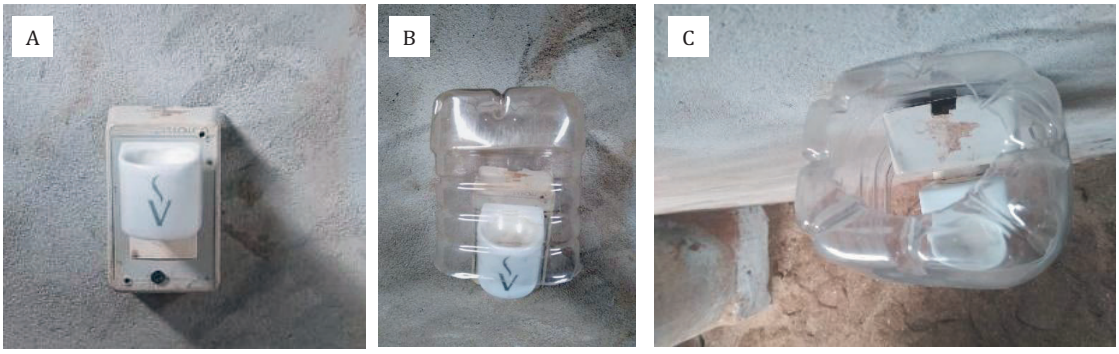
The average duration of the sessions was 30 minutes, of which 20 minutes were spent in the indoor arena and the remainder in the outdoor arena. The sessions were accompanied by professionals from specific areas, such as physiotherapists, psychologists, speech therapists and educators, who worked in multidisciplinary teams and devoted full attention to the patients.

2.3. Diffusion of lavender aromatherapy prior to equine-assisted therapy sessions

Aromatherapy was applied with lavender (*Lavandula augustifolia*) essential oil (manufacturer Florescer Alquimia, Estação, RS). The 50-mL bottle with pure essential oil was stored well capped, protected from heat and light and was the same used for all trials (same batch and origin).

A commercially available electric aroma air diffuser (Via Aroma, Porto Alegre, RS; 10-mL maximum capacity) was installed 1.2 m high from the bedding substrate in each of the two adjacent horse stalls (Figure 2). A shield protection around the diffusor was built with a 5-liter plastic container which was cut with 20-cm-diameter top and bottom holes to facilitate the diffusion of the lavender aroma around the stall environment.

The order of evaluation of the animals was random, respecting the schedule of equine-assisted therapy sessions predetermined by the multidisciplinary team in the Center, which had no relation to the study design or administration. The first three sessions of equine-assisted therapy were designated as a control treatment in which the animals in their stalls were exposed to a diffuser added to 5 mL of odorless drinking water, with no essential oil added, for 30 minutes. In the following three sessions, designated as aromatherapy treatment, a total of four drops of the 100% lavender essential oil was dissolved in 5 mL of water in the diffuser activated for 30 minutes to provide an environment with lavender aroma prior to the equine-assisted therapy sessions.



A - Front view of the air diffuser without protection; B - Front view of the air diffuser with plastic protection; C - Top view of the air diffuser showing the upper and lower holes in the shield to facilitate diffusion of the lavender essential oil for aromatherapy.

Figure 2 - Electric air aroma diffuser installed on the wall of the experimental stalls at 1.2 m high from the substrate bedding, near the horse´s head level. Equine Therapy Center of the Crioulo Horse, IFRS-Campus Sertão, Sertão, RS, Brazil.

During all periods described above, the horses had free movement in their respective stalls, being able to move away from or approach the diffuser at their free choice. At the end of the 30 minutes in the stall, the animal was calmly led by a handler to the preparation area where the animal was brushed and the saddle, girth and reins installed (average preparation time of 15 min). Afterwards, the horse was taken to the ramp for embarkation and disembarkation of the indoor arena, set up by the patient, and the equine-assisted therapy session was immediately started.

2.4. Behavioral assessment

The behavior of the horses was evaluated on two occasions during each trial, one during exposure to treatments in the stalls and the other during the development of equine-assisted therapy sessions held in the indoor arena.

Both stalls were equipped with video cameras attached to a 4-channel DVR system (Intelbras, São José, SC). The behaviors of the animals during the experiment were recorded on video for further evaluation. The behaviors presented in the ethogram are described in Table 1 were continuously evaluated (total evaluation time of 30 minutes pre-equine-assisted therapy session in the stalls).

Upon the beginning of the equine-assisted therapy session in the covered arena, each animal was evaluated by direct evaluation by the experimental observer for a period of 20-min (the total duration of the equine-assisted therapy session), and all sessions were filmed for later video analysis. The behavior observed during the sessions were continuously evaluated as shown in the ethogram presented in Table 2.

Table 1 - Ethogram used for behavioral assessment of horses during exposure to diffusion of water (control) and aromatherapy with lavender oil for a period of 30 minutes in the stalls, prior to the equine-assisted therapy sessions

Behaviors in the stall	Description
Head in the window	Horse with its head fully out the stall's window.
Head aligned with the diffuser	Horse with its head up aligned straight with the diffuser, looking directly at the diffuser.
Head down	Horse with its head down, relaxed and eyes half-open. Being approximately 1 m away from the diffuser.
Agitated	Horse in a state of restlessness, moving from one side to the other.
Others	All other behaviors performed that are not mentioned above.

Table 2 - Description of behaviors used for behavioral assessment during the equine-assisted therapy sessions lasting 20 minutes, after exposure to diffusion of water (control) and lavender oil in the stalls for 30 minutes in the stalls, prior to the sessions

Behaviors	Description
Balk	Horse ceases movement without being led to this action and resists continuing walking when encouraged by the handler.
Bite	Horse bites the halter or its handler (driver).
Lean on the embarkation	Horse pulls up to the boarding and disembark ramp with the session in progress, in an attempt to finish it (animals conditioned to this action).
Quit session	Horse turns towards the exit of the covered arena as a way of leaving the arena and ending the session.
Head-butts	Horse shakes it head from side to side, in some cases hitting its handler. Showing restlessness.

2.5. Horse handlers' perception of the horse's behavior during the equine-assisted therapy sessions

At the end of each equine-assisted therapy session, an objective questionnaire consisting of four closed questions was applied to the handler responsible for conducting the therapy horse that was under trial evaluation. The objective of the questionnaire was to assess the perception of the handlers who were familiarized with the behavioral characteristics of each of the horses at the Center. Thus, they had enough previous experience with the animals to be able to indicate possible changes in the behavior of the animals in preparation for the session and during the course of it. Handlers were fully blind to the experimental treatment to which the horse had been exposed prior to the start of the session and thus for responding to the questionnaire.

For each interview, the questionnaire (Table S1) was identified with the horse identification, the respondent´s name and the date of response. To question one, when the answer was "no", the respondent should indicate whether the animal behaved calmer or more agitated than normal. Incentives mentioned in question three were meant to either speed up or slow down the horse´s movement. They included oral stimuli (lip "kiss" sound and other voice commands) and handling incentives such as pulling the rein, leg movements from the patient and the handler's arm movements. The fourth and last question asked the respondents about the behavior of the horse during the preparation for the session, when placing the saddle and girth. From a total of 24 equine-assisted therapy sessions per treatment (total of 48 sessions), 16 surveys were collected from respondents following the control trials (water only diffused in the stalls; 8 responses were missing for control trials as handlers were unavailable after sessions to respond to the study). All 24 questionnaires employed to handlers after the aromatherapy sessions (lavender oil diffused with water in the stalls) were collected.

2.6. Statistical analyses

Behavioral variables observed during control and aromatherapy trials in the stall (in 30 minutes) and during the equine-assisted therapy session (in 20 minutes) were noted. The variables were assessed as total duration (initially recorded in seconds then transformed to minutes) and the respective frequencies shown during both evaluations, in the stalls and during the equine-assisted therapy sessions for further statistical analysis. The statistical model fitted is described as following:

$$y_{ij} = \mu + \alpha_i + \beta_j + e_{ij},$$

in which y_{ij} is the response variable measured in the j-th block (day) that received the i-th treatment (control and aromatherapy), μ is the general constant, α_i is the fixed effect of the i-th treatment, β_j is the random effect (animal) of the j-th block, and e_{ij} is the random error term.

The SAS Software 9.4 (SAS Institute, Inc., Cary, NC) was used for statistical analyses. Duration of each behavior (in minutes) observed was assessed for normality using the Shapiro-Wilk test (PROC UNIVARIATE, SAS Institute), no data transformation was required. Data were computed by analysis

of variance employing a mixed model (PROC MIXED, SAS Institute), the statistical model included the fixed treatment factors (control and aromatherapy), treatment nested on the test day (3 days/control and 3 days/treatment). Day was considered as a repeated factor in the model, while the animal was considered a random factor. Covariance structure (composite symmetry or first-order autoregressive symmetry) that best explained the variability of responses over time and the correlations between them was employed. Fixed main effects and interactions were tested and pair-wise comparisons were adjusted by Tukey's test. The frequency of behavior by treatment and per trial day for each treatment, as well as the handlers´ responses from the surveys were analyzed by Fisher´s exact test. Mean differences and frequencies with P-value < 0.05 were considered statistically different, and the exact P-values for respective variables are presented in the tables.

3. Results

3.1. Behavior in the stall

The exposure of horses to aromatherapy by diffusion with lavender oil for 30 minutes in the experimental stall resulted in a total time spent with the head down 21% greater when compared with exposure to the control treatment (P<0.05; Table 3). There was no evidence of an effect of the control and aromatherapy treatments on the behavior of head positioning in the stall window, head aligned with the diffuser, animal agitation, or other behavior (P>0.05; Table 3).

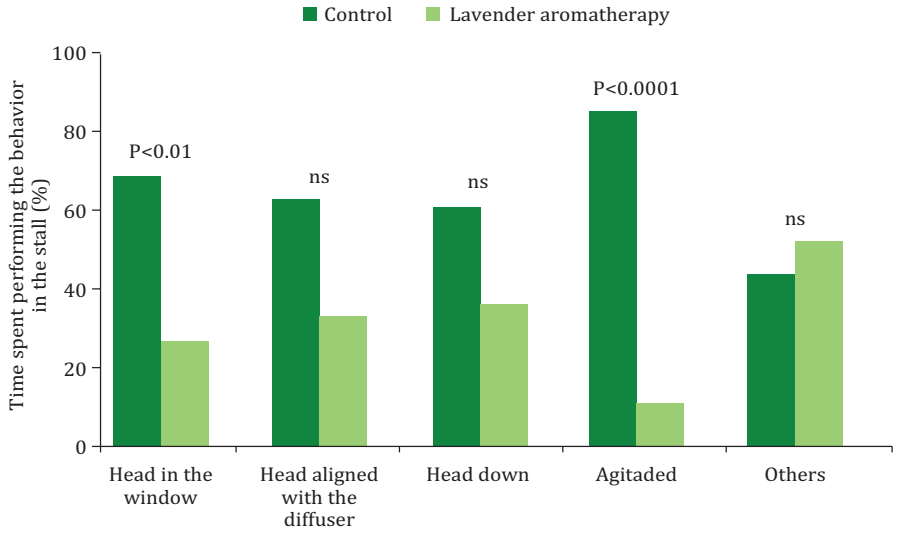
Table 3 - Total time in minutes spent by horses performing the behaviors in the stalls by exposure to a 30-minute diffusion of water only (control) or with lavender aromatherapy assessed by continuous observation

Time spent with the behavior in the stall (in minutes)	Treatment[1]		P-value
	Control	Aromatherapy	
Head down	21.2±1.3	26.9±1.4	0.003
Head in the window	7.0±1.6	5.5±2.0	0.561
Head aligned with the diffuser	0.45±0.2	0.5±0.2	0.840
Agitated	1.6±0.5	0.9±1.1	0.572
Others	1.4±0.4	1.5±0.4	0.867

[1] Each horse (n = 8) was evaluated on one day of the week, for a total of six weeks, with the first three evaluations as a control and the next three as aromatherapy.
Means ± standard error; statistical differences were considered at P<0.05.

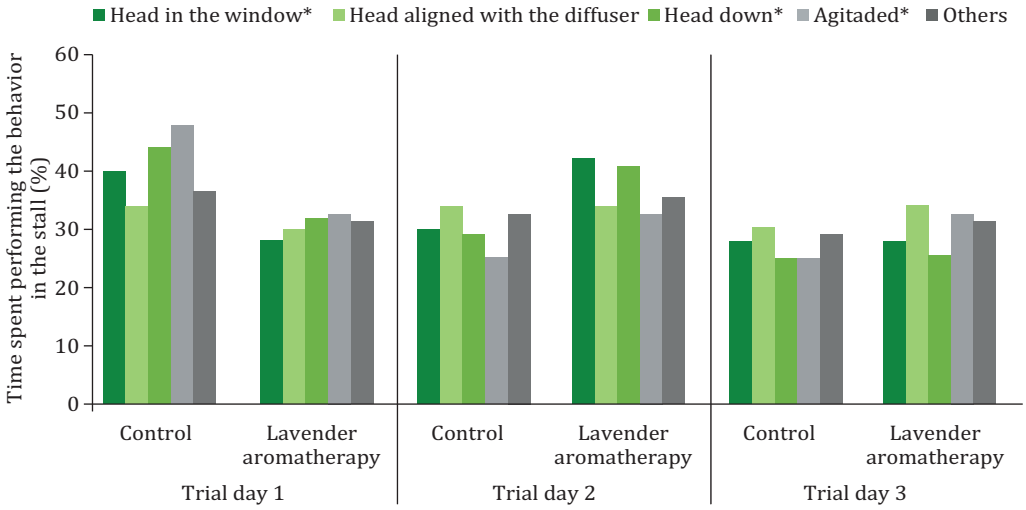
From the frequency analysis of behavior observed in the horse stalls shown in Figure 3, it was observed that, when exposed to aromatherapy, the animals showed a lower frequency of agitated behavior (12.5% for aromatherapy compared with 87.5% for control; P<0.0001) and of head positioning in the stall window (29.2% for aromatherapy compared with 65.2% for control; P<0.01). There was no statistical difference in head aligned with the diffuser (P = 0.06), head down, or behaviors grouped as "others" (P>0.1; Figure 3).

The results on the effect of the day nested within treatment on the frequencies of behavior in the stalls are shown in Figure 4. Head positioning in the window was more evident on day 1 of the control treatment, and on experimental day 2 for the aromatherapy treatment (P<0.05). Among the control test days, the frequency of agitation was lowest in the second test (P<0.05), while frequencies were similar to the days of exposure to aromatherapy (P>0.05; Figure 4). There were no statistical differences in the frequency of head behavior close to the diffuser, nor in "other behaviors" observed in relation to treatments and respective experimental days (P>0.05; Figure 4).



Each horse was evaluated on a weekday for a total of six weeks, with the first three evaluations as a control and the next three as aromatherapy treatment.
P<0.05 - statistical difference; ns - non-significant difference.

Figure 3 - Frequency of behaviors performed by horses in their housing stalls during exposure to water diffusion (control) and aromatherapy with lavender oil for a period of 30 minutes.



Each horse was evaluated on a weekday for a total of six weeks, with the first three evaluations as a control and the next three as aromatherapy treatment.
* Main effect of day (treatment) = P<0.05 for corresponding behaviors, noted in the legend.

Figure 4 - Frequency of behaviors performed by horses during exposure to water diffusion (control) and aromatherapy with lavender oil for a period of 30 minutes in the housing stalls.

3.2. Behavior during the equine-assisted therapy session

In the behavioral assessment conducted during the equine-assisted therapy sessions held in the covered arena for 20 minutes, it was observed that the total time spent performing head-butting behavior was reduced by half after exposure to aromatherapy (P<0.05; Table 4). The time spent

exhibiting the behaviors of biting, slamming, trying to quit the session and leaning on the embarkation and disembarkation ramp was not significantly affected by the lavender aromatherapy administered before the start of the sessions (P>0.05; Table 4).

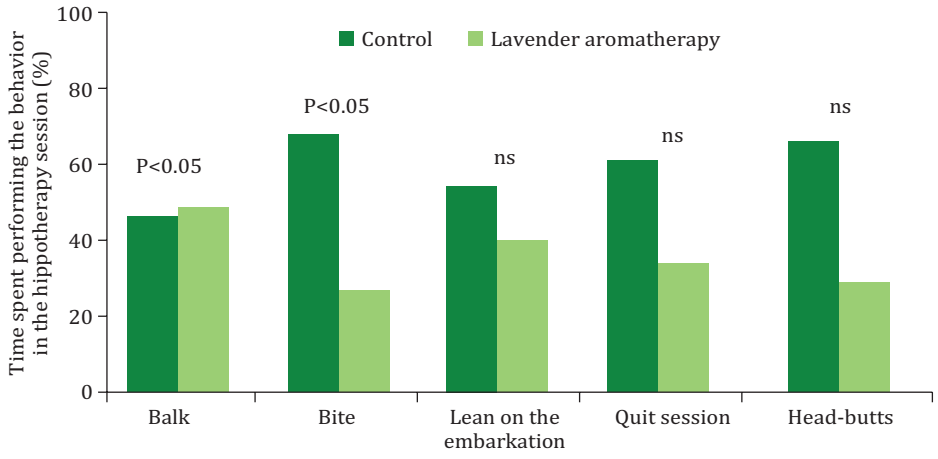
A day-related effect was observed only for the total time spent balking, which was greater on day 1 compared with assessment day 2 in the control treatment (0.4±0.2 min and 0.5±0.2 min, respectively; P<0.05); no balking behavior was observed in the control group for day 3, nor were any changes in behavioral times detected under aromatherapy (P>0.05; Figure 5).

During the equine-assisted therapy sessions, the frequency of balking was slightly higher in animals after exposure to aromatherapy than in the control treatment (51.4% vs. 48.6%, respectively, P<0.05; Figure 5). The behavior of biting or trying to bite the handler or the reins occurred at a 2.5-fold lower frequency in the aromatherapy treatment than to the control (P<0.05; Figure 5). Although numerically lower after exposure to aromatherapy, there was no statistical difference between treatments for the frequency of the horse head-butting the handler (P = 0.09), resting its head on the ramp where the patient boards and disembarks the animal (P = 0.07), and to yaw towards the exit of the covered arena as a way of exiting the session (P>0.01; Figure 5).

Table 4 - Total time spent by the horses performing the behaviors during the 20-minute equine-assisted therapy sessions in the covered arena after exposure to the lavender oil in the stalls for a period of 30 minutes

Time spent with the behavior during sessions (in minutes)	Treatment[1]		P-value
	Control	Aromatherapy	
Balk	0.4±0.2	0.5±0.2	0.497
Bite	0.7±0.6	0.4±1.4	0.670
Lean on the embarkation	0.09±0.02	0.12±0.02	0.406
Quit the session	0.13±0.02	0.08±0.02	0.120
Head-butts	0.14±0.01	0.06±0.01	0.015

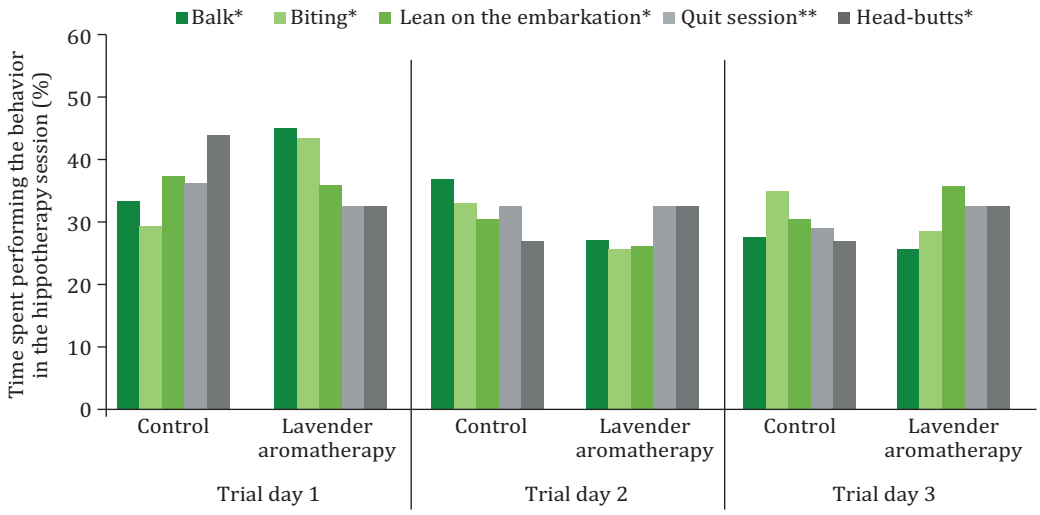
[1] Each horse (n = 8) was evaluated on one day of the week, for a total of six weeks, the first three as control and the next three as aromatherapy. Means ± standard error; statistical differences were considered at P<0.05.



Each horse was evaluated on a weekday for a total of six weeks, with the first three evaluations as a control and the next three as aromatherapy treatment.
P<0.05 - statistical difference; ns - non-significant difference.

Figure 5 - Frequency of behaviors performed by horses during hippotherapy sessions in the covered arena lasting 20 minutes, after exposure to water diffusion (control) and aromatherapy with lavender oil.

Regarding the frequency analysis of the day effect, both the frequency of getting stuck (P<0.01) and of biting the handler during the session (P<0.05) were higher on day 1 for horses exposed to aromatherapy and decreased over the following two days of treatment. The frequencies of balking were lower when compared with the control treatment values on the respective trial days (Figure 6). Horses relied less frequently on the ramp in attempt to terminate the session on the second day of exposure to aromatherapy, compared with the other days of treatment and in relation to the control treatment (P<0.05; Figure 6). The frequencies of deliberate head-butting on the manager during the equine-assisted therapy session were higher on the 1st day of the control treatment compared with the other days and were lower and constant after exposure to aromatherapy compared with the control (P<0.05; Figure 6). There was no day effect regarding the horses' attempts to quit the session through the arena gate (P = 0.06; Figure 6).



Each horse was evaluated on a day of the week, for a total of six weeks, with the first three evaluations as a control and the next three as aromatherapy.
Day (treatment) effect; for corresponding behaviors = *P<0.05 and **ns.

Figure 6 - Frequencies of behaviors performed by the horses during the hippotherapy sessions (20 minutes duration), right after exposure to water diffusion (control) and aromatherapy with lavender oil for a period of 30 minutes in the accommodation stalls according to three experimental days per treatment.

3.3. Handlers´ perception of equine behavior during equine therapy sessions

The horse handlers´ perception on their behavioral profile and session performance post treatments is summarized in Table 5. Handlers were fully unaware of the treatments to which horses were being exposed in the stalls (i.e. treatment-blind), before moving to the equine-assisted therapy sessions.

Regarding responses to question 1 – "Did the horse behave as usual during the session?" The handlers detected a change in the behavior of the horses during the equine-assisted therapy session (P<0.05) and attributed this change to the fact that they perceived the horses were calmer than normal, which became evident after exposure to aromatherapy (P<0.01; Table 5). When they were asked if the session flowed as expected, the respondents' perception was that the session flowed as expected for the center's routine, regardless of previous exposure to the treatments applied (P>0.05; Table 5). However, in response to question 3 – "Was it necessary to encourage the animal during the session? If so, it was

necessary", more respondents stated that it was necessary to offer minimal encouragement to horses when they were exposed to aromatherapy with lavender oil (P<0.05; Table 5). When asked whether horse behavior during saddling was as usual, handlers agreed that horses behaved as normal during the preparation for the equine-assisted therapy sessions, regardless of the treatment to which the horses were exposed, whether diffusion with water or with lavender oil (P>0.05; Table 5).

Table 5 - Perception of the handlers on the behavior of horses in the preparation and during the equine-assisted therapy sessions, after previous exposure to water diffusion (control) and diffusion of aromatherapy with lavender oil for 30 minutes in the stalls

Survey question to handlers	Yes to Control[1]	Yes to Aromatherapy[1]	P-value
1 - Did the horse behave as usual during the session? If not, was it:	93.7%	66.7%	0.043
Calmer than usual?	6.2%	50.0%	0.004
More agitated than usual?	12.5%	0.0%	0.154
2 - Did the session flow as expected?	93.7%	95.8%	0.492
3 - Was it necessary to encourage the animal during the session? If yes, it was necessary:	56.2%	70.8%	0.171
Little encouragement	12.5%	45.8%	0.025
Medium encouragement	37.5%	16.7%	0.100
Many encouragement	6.2%	8.3%	0.447
4 - Was the behavior when saddling the horse as usual? If not, what changed?	81.2%	83.3%	0.319
Showed wrong behavior	31.2%	16.7%	0.170
Stopped showing undesired behavior	6.2%	8.3%	0.447

[1] Each horse was evaluated on one day of the week for a total of six consecutive weeks, with the first three evaluations as a control and the next three as aromatherapy trials.
Statistical differences were considered at P<0.05.

4. Discussion

The application of aromatherapy with lavender essential oil in the stalls before the beginning of the equine-assisted therapy sessions reduced unwanted behaviors in horses both during exposure to aromatherapy and during the sessions. During the diffusion of aromatherapy, the animals stayed longer with their heads down, a posture considered relaxing for the species, and were less agitated. During the equine-assisted therapy sessions, there was a lower frequency of biting and head-butting toward the handlers who guided the animals. The handlers' perception was that, when previously exposed to aromatherapy with lavender oil, the therapy horses were calmer and needed little encouragement to move during the equine-assisted therapy sessions. The behavioral outcomes of aromatherapy contributed to easier handling and better progress of the equine-assisted therapy sessions, maximizing their benefits to the patients. Although the limited sample size of therapy horses used in the present study and the potential carryover effect from testing all animals first as control and later under lavender treatment may limit generalization, the outcomes are consistent with similar effects reported in the current scientific literature.

Aromatherapy with pure lavender essential oil dissolved in water was chosen for this study as it is one of the most common oils used for animals due to its calming effect (Heitman et al., 2018; Morag, 2018). During exposure to lavender aromatherapy for 30 minutes in the housing stall, horses reduced the frequency of most active behaviors. The therapy horses stood in a relaxing posture in the stall, with their head down 27 of the total 30 minutes, keeping their tail still or waving slowly, and occasionally

showing oral behaviors. Aromatherapy with lavender induced equine a relaxed facial expression and fewer spontaneous muscle contractures in the horses corroborating the physical relaxation outcome (Kosiara and Harrison, 2021). In agreement with the effects observed in the current study, lavender diffusion for 20 minutes before the horse´s exposure to stressors resulted in a reduced behavioral and physiological reactions such as alert posture, defecation, heart rate, and salivary cortisol concentration (Poutaraud et al., 2018).

Stress responses in horses are diagnosed through agitation, aggressiveness, and stereotypic behaviors performed in the stall (Waters et al., 2002). Yet, aromatherapy in the stalls led to at least twofold lower frequencies of "head in the stall window", "head aligned with the diffuser", "agitated", and "others". Horses that received aromatherapy placed their heads on the stall windows almost three times less often than the control group, suggesting that they chose to remain in the stall and were less anxious to leave. Similarly, "agitated" behavior in the stalls was about sevenfold less frequent, reinforcing the calming effect of the diffusion of lavender oil in the environment (Heitman et al., 2018; Morag, 2018; Silva et al., 2015). Air-diffused lavender essential oil for 15 minutes had a calming effect on seven horses subjected to an unpredictable acute stressor—an air horn sound—while they were resting in the stall, followed by a faster recovery of basal heart and respiratory rates (Ferguson et al., 2013). Even though horses may be familiar with their housing environment or conditions, limited social interaction or handling for specific practices, such as equine-assisted therapy sessions, may still be perceived as an adverse scenario for them (Young et al., 2012). The results of this study, combined with previous literature, reinforce the potential of aromatherapy as a beneficial tool to mitigate the effects on animals kept in indoor housing (i.e., stalls).

During the equine-assisted therapy sessions, although horses balked at a slightly higher frequency after exposure to the aromatherapy, the total time spent balking was not affected, and no complaints were reported by handlers regarding uncooperative horses. Reduced speed of movements may well be related to the muscle relaxation (Kosiara and Harrison, 2021; Morag, 2018) and even analgesic effects of the lavender (Silva et al., 2015). A study by Baldwin and Chea (2018) with nine horses (also used as their own controls) tested air diffused lavender and chamomile essential oils, dissolved in six drops of water, found that lavender consistently promoted relaxation of the autonomic nervous system, as indicated by lower heart rate variability (Baldwin and Chea, 2018).

When assessing horse behavior during the equine-assisted therapy, calmer horses in the stalls due to lavender aromatherapy bit the halter handle 2.5 times less often. They also showed less adverse interactions (biting and headbutting) with the handlers guiding them during the sessions and less frequent attempts to quit the session through the indoor arena gate. In fact, total time of headbutting was reduced by more than 50% with aromatherapy compared with that observed in the control treatment. The occurrence of undesired behavioral responses in horses hinders a smooth running of the therapy session, as for instance attempts to bite and headbutting can cause handler motor imbalance and even interrupt of the session. When selecting an animal for equine-assisted therapy, it is necessary to consider several questions, but the main one is behavioral profile of the horse (Waters et al., 2002; McDuffee et al., 2022).

The treatment-blinded handlers' perception corroborates the behavioral results observed in the horses while in the stall assessments and during the equine-assisted therapy sessions. Approximately 77% of handlers indicated that horses were calmer than normal during the equine-therapy sessions after aromatherapy exposure. Meanwhile, they had no issues with the routine of placing the saddle, girth, and bridle when preparing of the horses for the equine-assisted therapy sessions after the animals were exposed to aromatherapy. By reducing the frequency and duration of undesired behaviors during the sessions through the use of lavender aromatherapy, the therapy adds to the relevant benefits of equine-assisted intervention for patients (ANDE-Brasil, 2023; McDuffee et al., 2022).

Furthermore, improving equine welfare, especially for those used in animal-assisted therapy, goes beyond physical health (Sousa et al., 2025; Kaiser et al., 2006) or behavioral outcomes resulting from positive human-animal interactions and experiences (Kelly et al., 2021). Yet, for almost half of the handlers interviewed, the horses were calmer after aromatherapy and therefore required little

encouragement or incentive during the therapy sessions compared with the control treatment. In other words, in the absence of aromatherapy, the animals needed more verbal and physical interventions, which in turn could have been perceived as negative by the horses, to ensure a smooth course of the therapy (McDuffee et al., 2022). The verbal encouragements usually employed by the handlers at the equine-assisted therapy center were lip-smacking sounds ("kiss") and other vocal cues that the animals were familiar with. These sounds led the horses to increase or reduce their pace, or even stop their movement, according to the momentary need during the sessions. Pulling the halter more intensively, the exerciser's leg movements, and the handler's arm or lead-rope movements are considered physical incentives. Profiles of horses and patients, alongside the training and healthcare practices for horses engaged in equine-assisted therapy across Brazil, have shown that the animals' health and care can be limiting factors for therapy success (Sousa et al., 2025). In this sense, the handlers who routinely guided the horses during the equine-assisted therapy sessions were familiar with the animals, their routines, and their behavior.

Although horses used in equine therapy centers are adapted to the routine, they as sentient beings may occasionally perceive the relationship with their handler or even with the patient, sometimes unfamiliar, as an adverse event (McDuffee et al., 2022; Merkies and Franzin, 2021). An ethogram developed to identify stress-related behaviors in horses indicated that therapeutic riding for children and adolescents with intense or chronic vulnerability is more stressful for them, suggesting a decrease in the daily and weekly riding activity. Based on the data from this study, aromatherapy with lavender oil can be used as a tool to minimize this possible stressful effect of the environment in which the horse is inserted. Positive effects of lavender aromatherapy have also been shown to reduce stress response during transport of horses (Heitman et al., 2018). Lavender aromatherapy (20% oil in 80% water) used for pairs of eight horses, by diffusing it in the transport trailer for 15 min (8.8 km), yielded lower blood cortisol levels. The outcome of aromatherapy adds to expectations regarding the horse's behavioral profile of a good animal-assisted therapy as it becomes more tolerant to surrounding challenges and more easily accepts the activities proposed during the sessions. Thus, it contributes more effectively to the rehabilitation of humans with limitations or disabilities through equine-assisted therapy interventions.

5. Conclusions

Aromatherapy by air diffusion of lavender essential oil for 30 minutes in the stalls resulted in calmer horses used in equine-assisted therapy. Over the course of the therapy sessions, the horses previously exposed to aromatherapy slightly increased the frequency of balking but reduced by at least half the frequency of most undesired behaviors, such as attempting to quit the session, biting the halter or the riding guides, and head-butting handlers. As a result, the handlers perceived that, after lavender aromatherapy, the horses were calmer during the equine-assisted therapy sessions, with no negative impact on preparation for the sessions. The application of aromatherapy with lavender essential oil prior to the equine-assisted therapy sessions is proposed as a management tool to improve horse welfare and to aid in the success of therapy for patients.

Supplementary material

The supplementary material of this article can be found online at: https://www.rbz.org.br/wp-content/uploads/articles_xml/1806-9290-rbz-55-e20240233/1806-9290-rbz-55-e20240233-suppl01.pdf

Data availability

All content (raw and analyzed data, software codes, and respective video recordings) underlying the manuscript text is available upon request to the corresponding author.

Author contributions

Conceptualization: Facenda, T. and Poletto, R. **Data curation:** Facenda, T.; Oliveira, M. A. and Poletto, R. **Formal analysis:** Poletto, R. **Investigation:** Facenda, T. and Poletto, R. **Methodology:** Facenda, T.; Oliveira, M. A. and Poletto, R. **Project administration:** Facenda, T. and Poletto, R. **Resources:** Facenda, T. **Visualization:** Facenda, T. **Writing – original draft:** Facenda, T. and Poletto, R. **Writing – review & editing:** Facenda, T.; Oliveira, M. A. and Poletto, R.

Conflict of interest

The authors declare no conflict of interest.

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